L10 ANSWER 1 OF 11 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 2000433571 EMBASE
TITLE: Herpes simplex virus: Discovering the link between heparan sulphate and hereditary bone tumours.
AUTHOR: McCormick C.; Duncan G.; **Tufaro F.**
CORPORATE SOURCE: Dr. F. Tufaro, Dept. of Microbiology and Immunology, University of British Columbia, 6174 University Boulevard, Vancouver, BC V6T 1Z3, Canada. tufaro@interchange.ubc.ca
SOURCE: Reviews in Medical Virology, (2000) 10/6 (373-384).
Refs: 70
ISSN: 1052-9276 CODEN: RMVIEW
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 004 Microbiology
016 Cancer
033 Orthopedic Surgery
LANGUAGE: English
SUMMARY LANGUAGE: English

AB To gain entry into the host, viruses use host cell surface molecules that normally serve as receptors for other ligands. Herpes simplex virus type 1 (HSV-1) uses heparan sulphate (HS) glycosaminoglycans (GAGs) as receptors for initial attachment to the host cell surface. HS GAGs are both ubiquitous and structurally diverse, and normally serve as critical mediators of interactions between the cell and the extracellular environment. We have used the HS binding ability of HSV-1 to identify the function of a cellular gene, EXT1, which is involved in HS polymerisation.

Cellular factors that affect virus growth and replication are often key regulators of the cell cycle and EXT1 is no different - humans with inherited mutations in EXT1 have developmental defects that lead to bone tumours (hereditary multiple exostoses, HME) and sometimes chondrosarcomas. Thus, as a result of using HSV-1 as a molecular probe, a functionally orphaned disease gene now has a defined function. These findings highlight the utility of viruses for investigating important cellular processes. Copyright (C) 2000 John Wiley and Sons, Ltd.

L10 ANSWER 2 OF 11 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 1
ACCESSION NUMBER: 1999:614615 CAPLUS
DOCUMENT NUMBER: 131:327431
TITLE: Efficient infection of mature skeletal muscle with herpes simplex virus vectors by using dextran sulfate as a co-receptor
AUTHOR(S): Yeung, S. N.; Bockhold, K.; **Tufaro, F.**
CORPORATE SOURCE: Department of Microbiology and Immunology, University of British Columbia, Vancouver, BC, V6T 1Z3, Can.
SOURCE: Gene Ther. (1999), 6(9), 1536-1544
CODEN: GETHEC; ISSN: 0969-7128
PUBLISHER: Stockton Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The use of herpes simplex virus (HSV) vectors for gene delivery to skeletal muscle is hampered by a maturation-dependent loss of muscle fiber infectivity. Previous studies of HSV type 1 (HSV-1) infection in the rodent show that the loss of infectivity may be due, at least in part, to the development of the basal lamina throughout the course of maturation,

which may block the initial events in HSV infection. To initiate infection, HSV normally attaches to cell surface heparan sulfate, which stabilizes the virus such that it can interact with secondary protein receptors required for entry into host cells. In this study, we demonstrate that heparan sulfate biosynthesis is down-regulated during skeletal muscle maturation. When myofibers were treated with a variety of enzymes, including collagenase type IV or chondroitin ABC lyase, HSV infection was restored, which suggests that virus secondary receptors were present but not readily accessible to the virus in the intact myofiber. Surprisingly, we also found that HSV-1 infectivity could be restored in vitro and in vivo by exposing myofibers to low concns. of the **glycosaminoglycan** analog dextran sulfate, which appears to act as a surrogate receptor to stabilize the virus at the myofiber surface such that HSV can engage addnl. receptors. This demonstration that the basal lamina is not an abs. block to HSV-1 infection is remarkable because it allows for the nondestructive targeting of HSV-1 to mature myofibers and greatly expands the usefulness of HSV as a gene therapy vector for the treatment of inherited and acquired diseases.

REFERENCE COUNT: 66
REFERENCE(S):
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ALL CITATIONS AVAILABLE IN THE RE FORMAT

L10 ANSWER 3 OF 11 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 1999365573 EMBASE
TITLE: New perspectives on the molecular basis of hereditary bone tumours.
AUTHOR: McCormick C.; Duncan G.; **Tufaro F.**
CORPORATE SOURCE: C. McCormick, Dept. of Microbiology/Immunology, University of British Columbia, Vancouver, BC V6T 1Z3, Canada. tufaro@interchange.ubc.ca
SOURCE: Molecular Medicine Today, (1999) 5/11 (481-486).
Refs: 53
ISSN: 1357-4310 CODEN: MMTOFK
PUBLISHER IDENT.: S 1357-4310(99)01593-2
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 016 Cancer
022 Human Genetics
033 Orthopedic Surgery
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Bone development is a highly regulated process sensitive to a wide variety of hormones, inflammatory mediators and growth factors. One of the most common hereditary skeletal dysplasias, hereditary multiple exostoses (HME), is an autosomal dominant disorder characterized by skeletal malformations that manifest as bony, benign tumours near the end of long bones. HME is usually caused by defects in either one of two genes, EXT1 and EXT2, which encode enzymes that catalyze the biosynthesis of heparan sulphate, an important component of the extracellular matrix. Thus, HME-linked bone tumours, like many other skeletal dysplasias, probably result from disruptions in cell surface architecture. However, despite the recent success in unravelling functions for several members of the EXT gene family, significant challenges remain before this knowledge can be used to develop new approaches for the diagnosis and treatment of disease.

L10 ANSWER 4 OF 11 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 2
ACCESSION NUMBER: 1998:672025 CAPLUS

DOCUMENT NUMBER: 130:64007
TITLE: The putative tumor suppressors EXT1 and EXT2 are glycosyltransferases required for the biosynthesis of heparan sulfate
AUTHOR(S): Lind, Thomas; **Tufaro, Frank**; McCormick, Craig; Lindahl, Ulf; Lidholt, Kerstin
CORPORATE SOURCE: Department of Medical Biochemistry and Microbiology, The Biomedical Center, Uppsala University, Uppsala, S-751 23, Swed.
SOURCE: J. Biol. Chem. (1998), 273(41), 26265-26268
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Hereditary multiple exostoses, characterized by multiple cartilaginous tumors, is ascribed to mutations at three distinct loci, denoted EXT1-3. Here, the authors report the purifn. of a protein from bovine serum that harbored the D-glucuronyl (GlcA) and N-acetyl-D-glucosaminyl (GlcNAc) transferase activities required for biosynthesis of the **glycosaminoglycan**, heparan sulfate (HS). This protein was identified as EXT2. Expression of EXT2 yielded a protein with both glycosyltransferase activities. Moreover, EXT1, previously found to rescue defective HS biosynthesis (McCormick, C., Leduc, Y., Martindale, D., Mattison, K., Esford, L. E., Dyer, A. P., and Tufaro, F. (1998) Nat. Genet. 19, 158-161), was shown to elevate the low GlcA and GlcNAc transferase levels of mutant cells. Thus at least two members of the EXT family of tumor suppressors encode glycosyltransferases involved in the chain elongation step of HS biosynthesis.
REFERENCE COUNT: 35
REFERENCE(S): (1) Ahn, J; Nat Genet 1995, V11, P137 CAPLUS
(2) Bellaiche, Y; Nature 1998, V394, P85 CAPLUS
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(5) Gruenheid, S; J Virol 1993, V67, P93 CAPLUS
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ALL CITATIONS AVAILABLE IN THE RE FORMAT

L10 ANSWER 5 OF 11 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 3
ACCESSION NUMBER: 1998:289275 CAPLUS
DOCUMENT NUMBER: 129:65866
TITLE: Analysis of CD44 interactions with hyaluronan in murine L cell fibroblasts deficient in **glycosaminoglycan** synthesis: a role for chondroitin sulfate
AUTHOR(S): Esford, Lesley E.; Maiti, Arpita; Bader, Sharon A.; **Tufaro, Frank**; Johnson, Pauline
CORPORATE SOURCE: Department of Microbiology and Immunology, University of British Columbia, Vancouver, BC, V6T 1Z3, Can.
SOURCE: J. Cell Sci. (1998), 111(7), 1021-1029
CODEN: JNCSAI; ISSN: 0021-9533
PUBLISHER: Company of Biologists Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB CD44 is a widely expressed cell adhesion mol. that binds the extracellular matrix component, hyaluronan, in a tightly regulated manner. Previous studies have shown that the CD44-hyaluronan interaction is affected by changes in the glycosylation state of CD44. Here, the authors take advantage of several well-characterized murine L cell mutants defective in heparan sulfate synthesis (gro2C cells), heparan sulfate and chondroitin sulfate synthesis (sog9 cells), and **glycosaminoglycan** and oligosaccharide processing (sog8 cells) to assess the effects of these defects on the hyaluronan binding ability of CD44. In parental L cells and gro2C cells, CD44 was induced to bind hyaluronan after addn. of the activating, anti-CD44 monoclonal antibody, IRAWB 14. By contrast, no

inducible binding was obsd. in sog9 cells. Treatment of L cells with sodium chlorate, an inhibitor of sulfation, also abolished inducible hyaluronan binding. However, inducible and some constitutive hyaluronan binding was obsd. in sog8 cells. Thus, sulfation and, in particular, the addn. of chondroitin sulfate are required for inducible hyaluronan binding by CD44 in L cells. However, in the absence of fully processed oligosaccharides, chondroitin sulfate is not essential for hyaluronan binding, indicating that the effect of chondroitin sulfate is dependent upon the glycosylation state of the cell. Thus, in addn. to glycosylation, chondroitin sulfate biosynthesis is an important post-translational modification that can affect the hyaluronan binding ability of CD44.

L10 ANSWER 6 OF 11 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1998:564861 CAPLUS
DOCUMENT NUMBER: 129:274171
TITLE: The putative tumor suppressor EXT1 alters the expression of cell-surface heparan sulfate
AUTHOR(S): McCormick, Craig; Leduc, Yves; Martindale, Diane; Mattison, Kirsten; Esford, Lesley E.; Dyer, Angela P.; **Tufaro, Frank**
CORPORATE SOURCE: Dep. Microbiology & Immunology, Univ. British Columbia, Vancouver, V6T 1Z3, Can.
SOURCE: Nat. Genet. (1998), 19(2), 158-161
CODEN: NGENEC; ISSN: 1061-4036
PUBLISHER: Nature America
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Hereditary multiple exostoses (HME) is an autosomal dominant disorder characterized by the formation of cartilage-capped tumors (exostoses) that develop from the growth plate of endochondral bone. This condition can lead to skeletal abnormalities, short stature and malignant transformation of exostoses to chondrosarcomas or osteosarcomas. Linkage analyses have identified three different genes for HME, EXT1 on 8q24.1, EXT2 on 11p11-13 and EXT3 on 19p (refs. 6-9). Most HME cases have been attributed to missense or frameshift mutations in these tumor-suppressor genes, whose functions have remained obscure. Here, we show that EXT1 is an ER-resident type II transmembrane glycoprotein whose expression in cells results in the alteration of the synthesis and display of cell surface heparan sulfate glycosaminoglycans (GAGs). Two EXT1 variants contg. etiol. missense mutations failed to alter cell-surface glycosaminoglycans, despite retaining their ER-localization.

L10 ANSWER 7 OF 11 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 4
ACCESSION NUMBER: 1996:759233 CAPLUS
DOCUMENT NUMBER: 126:73239
TITLE: Dextran sulfate can act as an artificial receptor to mediate a type-specific herpes simplex virus infection via glycoprotein B
AUTHOR(S): Dyer, Angela P.; Banfield, Bruce W.; Martindale, Diane; Spannier, Dell-Marie; **Tufaro, Frank**
CORPORATE SOURCE: Dep. Microbiol. Immunol., Univ. British Columbia, Vancouver, BC, V6T 1Z3, Can.
SOURCE: J. Virol. (1997), 71(1), 191-198
CODEN: JOVIAM; ISSN: 0022-538X
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Herpes simplex virus (HSV) adsorption to host cells in mediated, at least

in part, by the interaction of viral glycoproteins with cell surface glycosaminoglycans such as heparan sulfate and chondroitin sulfate. To investigate the contribution of various cell surface components in the infection pathway, the authors isolated a mutant cell line, sog9, which is unable to synthesize glycosaminoglycans. Although HSV-1 and HSV-2 infection of sog9 cells is diminished, the cells are still infected at about 0.5% efficiency, which suggests that these cells normally express at least one nonglycosaminoglycan receptor. In this report, the authors used sog9 cells to test whether **glycosaminoglycan** analogs, such as dextran sulfate (DS), could functionally substitute for cellular glycosaminoglycans to initiate HSV infection. The authors show that high-mol.-wt. DS added either prior to or during inoculation stimulated HSV-1 but not HSV-2 infection by up to 35-fold; DS added after viral adsorption had no effect on infection efficiency. Moreover, DS stimulated HSV-1 infection at 4.degree., indicating that this compd. impinged on an early, energy-independent step in infection. Using radiolabeled virus, the authors showed that HSV-1 is more efficient than HSV-2 in adsorbing to DS immobilized on microtiter wells. This raised the possibility that only HSV-1 could engage addnl. receptors to initiate infection in the presence of DS. To det. which viral component(s) facilitated DS stimulation, a panel of intertypic recombinants and deletion mutant viruses was investigated. These assays showed that DS stimulation of infection is mediated primarily by gB-1. Thus, this study provides direct evidence that a principal role for cell surface glycosaminoglycans in HSV infection is to provide an efficient matrix for virus adsorption. Moreover, by using DS as an alternative adsorption matrix (a trans receptor), the authors uncovered a functional, type-specific interaction of HSV-1 with a cell surface receptor.

L10 ANSWER 8 OF 11 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 5

ACCESSION NUMBER: 1995:568156 CAPLUS
DOCUMENT NUMBER: 122:287890
TITLE: Cell surface proteoglycans are not essential for infection by pseudorabies virus
AUTHOR(S): Karger, Axel; Saalmueller, Armin; **Tufaro, Frank**; Banfield, Bruce W.; Mettenleiter, Thomas C.
CORPORATE SOURCE: Institute of Molecular and Cellular Virology, Friedrich Loeffler Institutes, Tuebingen, D-72076, Germany
SOURCE: J. Virol. (1995), 69(6), 3482-9
CODEN: JOVIAM; ISSN: 0022-538X
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Cell surface proteoglycans, in particular those carrying heparan sulfate glycosaminoglycans, play a major role in primary attachment of herpesviruses to target cells. In pseudorabies virus (PrV), glycoprotein gC has been shown to represent the major heparan sulfate-binding virion envelope protein. Since PrV gC is nonessential for viral infectivity in vitro and in vivo, either the interaction between virion envelope and cellular heparan sulfate is not necessary to mediate infection or other virion envelope proteins can substitute as heparan sulfate-binding components in the absence of gC. To answer these questions, the authors analyzed the infectivity of isogenic gC+ and gC- PrV on mouse L-cell derivs. with defects in **glycosaminoglycan** biosynthesis, using a rapid and sensitive fluorescence-based .beta.-galactosidase assay and single-cell counting in a fluorescence-activated cell sorter. The data show that (i) in the virion, glycoprotein gC represents the only proteoglycan-binding envelope protein, and (ii) cellular proteoglycans are

not essential for infectivity of PrV. Attachment studies using radiolabeled virions lacking either gC or the essential gD confirmed these results and demonstrated that PrV gD mainly contributes to binding of Pr virions to cell surface components other than proteoglycans. These data demonstrate the presence of a proteoglycan-independent mode of attachment for Pr virions leading to infectious entry into target cells.

L10 ANSWER 9 OF 11 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 6
ACCESSION NUMBER: 1995:568135 CAPLUS
DOCUMENT NUMBER: 122:310180
TITLE: Sequential isolation of proteoglycan synthesis mutants by using herpes simplex virus as a selective agent: evidence for a proteoglycan-independent virus entry pathway
AUTHOR(S): Banfield, Bruce W.; Leduc, Yves; Esford, Lesley; Schubert, Kathryn; **Tufaro, Frank**
CORPORATE SOURCE: Dep. Microbiol. Immunology, Univ. British Columbia, Vancouver, V6T 1Z3, Can.
SOURCE: J. Virol. (1995), 69(6), 3290-8
CODEN: JOVIAM; ISSN: 0022-538X
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A novel mouse L-cell mutant cell line defective in the biosynthesis of glycosaminoglycans was isolated by selection for cells resistant to herpes simplex virus (HSV) infection. These cells, termed sog9, were derived from mutant parental gro2C cells, which are themselves defective in heparan sulfate biosynthesis and 90% resistant to HSV type 1 (HSV-1) infection compared with control L cells (S. Gruenheid et al., 1993). Here the authors show that sog9 cells exhibit a 3-order-of-magnitude redn. in susceptibility to HSV-1 compared with control L cells. In steady-state labeling expts., sog9 cells accumulated almost no [35S]sulfate-labeled or [6-3H]glucosamine-labeled glycosaminoglycans, suggesting that the initiation of **glycosaminoglycan** assembly was specifically reduced in these cells. Despite these defects, sog9 cells were fully susceptible to vesicular stomatitis virus (VSV) and permissive for both VSV and HSV replication, assembly, and egress. HSV plaques formed in the sog9 monolayers in proportion to the amt. of input virus, suggesting the block to infection was in the virus entry pathway. More importantly, HSV-1 infection of sog9 cells was not significantly reduced by sol. heparan sulfate, indicating that infection was **glycosaminoglycan** independent. Infection was inhibited by sol. gD-1, however, which suggests that glycoprotein gD plays a role in the infection of this cell line. The block to sog9 cell infection by HSV-1 could be eliminated by adding sol. dextran sulfate to the inoculum, which may act by stabilizing the virus at the sog9 cell surface. Thus, sog9 cells provide direct genetic evidence for a proteoglycan-independent entry pathway for HSV-1, and results with these cells suggest that HSV-1 is a useful reagent for the direct selection of novel animal cell mutants defective in the synthesis of cell surface proteoglycans.

L10 ANSWER 10 OF 11 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 7
ACCESSION NUMBER: 1995:559257 CAPLUS
DOCUMENT NUMBER: 122:312024
TITLE: Evidence for an interaction of herpes simplex virus with chondroitin sulfate proteoglycans during infection
AUTHOR(S): Banfield, Bruce W.; Leduc, Yves; Esford, Lesley; Visalli, Robert J.; Brandt, Curtis R.; **Tufaro, Frank**
CORPORATE SOURCE: Dep. Microbiol. Immunol., Univ. British Columbia, Vancouver, BC, V6T 1Z3, Can.
SOURCE: Virology (1995), 208(2), 531-9

CODEN: VIRLAX; ISSN: 0042-6822
DOCUMENT TYPE: Journal
LANGUAGE: English
AB In a previous study, a mouse L cell mutant was isolated which is 90% resistant to HSV-1 infection (S. Gruenheid, L. Gatzke, H. Meadows, and F. Tufaro. J. Virol. 67, 93-100, 1993). This cell line, termed gro2C, failed to express heparan sulfate (HS) glycosaminoglycans on the cell surface, which normally act as initial receptors for HSV-1 attachment to cultured cells. In this report, we extended the characterization of gro2C cells to explore the possibility that cell-surface chondroitin sulfate (CS) facilitates virus attachment to gro2C cells in the absence of HS. We found that sol. CS types A, B, and C strongly interfere with adsorption of HSV-1 to the surface of gro2C cells in a dose-dependent manner, and CS type B (dermatan sulfate) inhibited adsorption to parental (control) L cells by up to 10%. Moreover, gro2C cell infection was hypersensitive to inhibition by HS in comparison to control L cell infection. In all cases, a decrease in adsorption resulted in a decrease in infection. By contrast, the highly-sulfated **glycosaminoglycan** analog dextran sulfate was a relatively poor inhibitor of gro2C cell infection, indicating that the inhibitory effects of CS were related to its carbohydrate structure and not solely to its strong neg. charge. By using a mutant virus strain which does not express the heparin-binding glycoprotein gC, we show that gC was not required for infection of gro2C cells, and was not required for the inhibition by HS or CS. Thus, the characterization of gro2C cell infection has revealed that one or more components of the HSV-1 particle can interact with cell-surface CS as well as HS to mediate infection of susceptible cells.

L10 ANSWER 11 OF 11 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 93002968 EMBASE
DOCUMENT NUMBER: 1993002968
TITLE: Herpes simplex virus infection and propagation in a mouse L cell mutant lacking heparan sulfate proteoglycans.
AUTHOR: Gruenheid S.; Gatzke L.; Meadows H.; **Tufaro F.**
CORPORATE SOURCE: Microbiology/Immunology Department, University of British Columbia,Vancouver, BC V6T 1Z3, Canada
SOURCE: Journal of Virology, (1993) 67/1 (93-100).
ISSN: 0022-538X CODEN: JOVIAM
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
026 Immunology, Serology and Transplantation
LANGUAGE: English
SUMMARY LANGUAGE: English
AB We have isolated a variant line of mouse L cells, termed gro2C, which is partially resistant to infection by herpes simplex virus type 1 (HSV-1). Characterization of the genetic defect in gro2C cells revealed that this cell line harbors a specific defect in the heparan sulfate synthesis pathway. Specifically, anion-exchange high-performance liquid chromatography of metabolically radiolabeled glycosaminoglycans indicated that chondroitin sulfate moieties were synthesized normally in the mutant cells, whereas heparin-like chains were absent. Because of these properties, we have used these cells to investigate the role of heparan sulfate proteoglycans in the HSV-1 life cycle. In this report, we demonstrate that the partial block to HSV-1 infection in gro2C cells occurs in the virus entry pathway. Virus adsorption assays using radiolabeled HSV-1 (KOS) revealed that the gro2C cell surface is a relatively poor target for HSV-1 in that virus attachment was 85% lower in

the mutant cells than in the parental L cell controls. A portion of the 15% residual virus adsorption was functional, however, insofar as gro2C cells were susceptible to HSV-1 infection in plaque assays and in single-step growth experiments. Moreover, although the number of HSV-1 plaques that formed in gro2C monolayers was reduced by 85%, the plaque morphology was normal, and the virus released from the mutant cells was infectious. Taken together, these results provide strong genetic evidence that heparan sulfate proteoglycans enhance the efficiency of HSV attachment to the cell surface but are otherwise not essential at any stage of the lytic cycle in culture. Moreover, in the absence of heparan sulfate, other cell surface molecules appear to confer susceptibility to HSV, leading to a productive viral infection.

drewwang

=> s glycosaminoglycan and transfection

L11 223 GLYCOSAMINOGLYCAN AND TRANSFECTION

=> s l11 and vir?

L12 23 L11 AND VIR?

=> dup rem l12

PROCESSING COMPLETED FOR L12
L13 13 DUP REM L12 (10 DUPLICATES REMOVED)

=> d 1- ibib abs

YOU HAVE REQUESTED DATA FROM 13 ANSWERS - CONTINUE? Y/(N):y

L13 ANSWER 1 OF 13 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 1
ACCESSION NUMBER: 2001:150128 CAPLUS
DOCUMENT NUMBER: 135:3852
TITLE: Contributions of gD receptors and **glycosaminoglycan** sulfation to cell fusion mediated by herpes simplex **virus** 1
AUTHOR(S): Terry-Allison, T.; Montgomery, R. I.; Warner, M. S.; Geraghty, R. J.; Spear, P. G.
CORPORATE SOURCE: Department of Microbiology-Immunology, Northwestern University Medical School, Chicago, IL, 60611-3010, USA
SOURCE: Virus Res. (2001), 74(1-2), 39-45
CODEN: VIREDF; ISSN: 0168-1702
PUBLISHER: Elsevier Science Ireland Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Two cell surface proteins (nectin-1/HveC and nectin-2/HveB) serve as receptors for the entry of herpes simplex **virus** 1 (HSV-1). Wild-type and/or mutant strains of these cell surface proteins were also found to serve as receptors for HSV-1-induced cell fusion. **Transfection** with genomic DNA from a syncytial HSV-1 strain encoding wild-type gD resulted in fusion of Chinese hamster ovary (CHO) cells expressing nectin-1 but not of cells expressing nectin-2. In contrast, **transfection** with DNA from a related HSV-1 strain encoding the mutant Rid1 form of gD resulted in fusion of CHO cells expressing either receptor but not of control cells. These results are consistent with the ability of each receptor to mediate entry of **viruses** expressing wild-type or Rid1 gD and with results obtained previously with HVEM (HveA), a third HSV-1 entry receptor. Undersulfation of GAGs in receptor-expressing cell lines predictably reduced susceptibility to HSV-1 infection. In contrast, susceptibility to cell fusion mediated by HVEM or nectin-1 was not reduced. Undersulfation of GAGs partially inhibited cell fusion mediated by nectin-2. Thus, HSV-1-induced cell fusion requires a gD-binding entry receptor, the ability of an HSV-1 strain to use HVEM, nectin-2 or nectin-1 for cell fusion depends on the allele of gD expressed, and GAGs may influence cell fusion, dependent on the gD-binding receptor used, but are less important for cell fusion mediated by HVEM, nectin-2 or nectin-1 than for **viral** entry.

REFERENCE COUNT: 37
REFERENCE(S): (1) Banfield, B; J Virol 1995, V69, P3290 CAPLUS
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(4) Draper, K; J Virol 1984, V51, P578 CAPLUS
(5) Feyzi, E; J Biol Chem 1997, V272, P24850 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 2 OF 13 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2000:616365 CAPLUS
DOCUMENT NUMBER: 133:218505
TITLE: Introduction of an N-glycosylation cassette into proteins at random sites and cDNA library construction
: expression of neo-glycosylated FGF
INVENTOR(S): Imamura, Toru; Asada, Masahiro; Suzuki, Satoru; Oka, Shuichi; Yoneda, Atsuko
PATENT ASSIGNEE(S): Agency for Industrial Science and Technology, Japan
SOURCE: Jpn. Kokai Tokkyo Koho, 36 pp.
CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 2000236880	A2	20000905	JP 1999-41538	19990219

AB A method of prodn. for a cDNA library of genes coding for mutant proteins with amino acid or peptide inserted at random sites, is disclosed. Addn. of extra nucleotides at either 5' or 3' end of DNA coding for an amino acid or peptide allows insertion into the gene coding for a protein in frame, ie., without disrupting the reading frame. DNA coding for an amino acid or peptide may be linked to a marker gene for selection of transformants. Preferably, the amino acid or peptide contains glycosylation sites for sulfated oligosaccharide, **glycosaminoglycan**, N-linked saccharide, or O-linked saccharide. FGF family or heparin binding growth factor proteins such as FGF-1a, FGF-6/1a, or secFGF-1 are used. The authors developed a method for introducing an N-glycosylation cassette into proteins at random sites by constructing cDNAs and expressing it in mammalian cells. The protocol entails four steps: (i) generation of cDNAs that contain single, randomly-located blunt end cuts: (ii) ligation of N-glycosylation cassettes into the blunt end cuts in three-frame formats: (iii) selection of the cDNA clones encoding N-glycosylated proteins; and (iv) subcloning into an expression vector for **transfection** and expression in mammalian cells. This method was evaluated using secreted fibroblast growth factor (FGF) as a model protein. Several secreted FGF cDNA clones, each contg. an AsnLeuSer-coding sequence at a random site, were obtained. When these clones were expressed in mammalian cells, some of the secreted FGFs were N-glycosylated. The method described here should also be applicable for random introduction of functional oligopeptide/polypeptide cassettes into **virtually** any protein of interest.

L13 ANSWER 3 OF 13 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 2
ACCESSION NUMBER: 2001:14890 CAPLUS
DOCUMENT NUMBER: 134:168222
TITLE: Examination of the biophysical interaction between plasmid DNA and the polycations, polylysine and polyornithine, as a basis for their differential gene **transfection** in-vitro
AUTHOR(S): Ramsay, Euan; Hadgraft, Jon; Birchall, James; Gumbleton, Mark
CORPORATE SOURCE: Pharmaceutical Cell Biology, Welsh School of Pharmacy,

Cardiff University, Cardiff, CF10 3XF, UK
SOURCE: Int. J. Pharm. (2000), 210(1-2), 97-107
CODEN: IJPHDE; ISSN: 0378-5173
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The impetus to develop non-**viral** gene delivery vectors has led to examn. of synthetic polycationic polymers as plasmid DNA (pDNA) condensing agents. Previous reports have highlighted superiority (up to .times. 10-fold) in the in-vitro **transfection** of pDNA complexes formed by poly-(L)-ornithine (PLO) compared to those formed with poly-(L)-lysine (PLL). The apparent basis for this consistent superiority of PLO complexes remains to be established. This comparative study investigates whether physico-chem. differences in the supramol. properties of polycation:pDNA complexes provide a basis for their obsd. differential gene **transfection**. Specifically, particle size distribution and zeta potential of the above complexes formulated over a wide range of polycation:pDNA ratios were found to be consistent with a condensed (150-200 nm) cationic (+ 30-40 mV) system but not influenced by the type of cationic polymer used. A spectrofluorimetric EtBr exclusion assay showed that polycation:pDNA complexes display different pDNA condensation behavior, with PLO able to condense pDNA at a lower polycation mass compared to both polylysine isomers, and form complexes that were more resistant to disruption following challenge with anionic counter species, i.e. poly-(L)-aspartic acid and the **glycosaminoglycan** mol., heparin. We conclude that particle size and surface potential as gross supramol. properties of these complexes do not represent, at least in a non-biol. system, the basis for the differential **transfection** behavior obsd. between these condensing polymers. However, differences in the ability of the polylysine and polyornithine polymers to interact with pDNA and to stabilize the polymer-pDNA assembly could have profound effects upon the cellular and sub-cellular biol. processing of pDNA mols. and contribute to the disparity in cell **transfection** efficiency obsd. between these complexes.

REFERENCE COUNT: 33
REFERENCE(S):
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ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 4 OF 13 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER: 1999:375419 SCISEARCH
THE GENUINE ARTICLE: 194NL
TITLE: Chinese hamster ovary cell mutants defective in **glycosaminoglycan** assembly and glucuronosyltransferase I
AUTHOR: Bai X M; Wei G; Sinha A; Esko J D (Reprint)
CORPORATE SOURCE: UNIV CALIF SAN DIEGO, DEPT MED, DIV CELLULAR & MOL MED, GLYCOBIOL PROGRAM, 9500 GILMAN DR, CMM E 1055, LA JOLLA, CA 92093 (Reprint); UNIV CALIF SAN DIEGO, DEPT MED, DIV CELLULAR & MOL MED, GLYCOBIOL PROGRAM, LA JOLLA, CA 92093
COUNTRY OF AUTHOR: USA
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (7 MAY 1999) Vol. 274, No. 19, pp. 13017-13024.
Publisher: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814.
ISSN: 0021-9258.
DOCUMENT TYPE: Article; Journal

FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 86
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The proteoglycans of animal cells typically contain one or more heparan sulfate or chondroitin sulfate chains. These glycosaminoglycans assemble on a tetrasaccharide primer, -GlcA beta 1,3Gal beta 1,3Gal beta 1,4Xyl beta-O-, attached to specific serine residues in the core protein. Studies of Chinese hamster ovary cell mutants defective in the first or second enzymes of the pathway (xylosyl-transferase and galactosyltransferase I) show that the assembly of the primer occurs by sequential transfer of single monosaccharide residues from the corresponding high energy nucleotide sugar donor to the non-reducing end of the growing chain. In order to study the other reactions involved in linkage tetrasaccharide assembly, we have devised a powerful selection method based on induced resistance to a mitotoxin composed of basic fibroblast growth factor-saporin. One class of mutants does not incorporate (SO4)-S-35 and [6-H-3]GlcN into **glycosaminoglycan** chains. Incubation of these cells with naphthol-beta-D-xyloside (Xyl beta-O-Np) resulted in accumulation of linkage region intermediates containing 1 or 2 mol of galactose (Gal beta 1, 4Xyl beta-O-Np and Gal beta 1, 3Gal beta 1, 4Xyl beta-O-Np) and sialic acid (Sia alpha 2,3Gal beta 1, 3Gal beta 1, 4Xyl beta-O-Np) but not any GlcA-containing oligosaccharides. Extracts of the mutants completely lacked UDP-glucluronic acid:Gal beta 1,3Gal-R glucuronosyltransferase (GlcAT-I) activity, as measured by the transfer of GlcA from UDP-GlcA to Gal beta 1,3Gal beta-O- naphthalenemethanol (<0.2 versus 3.6 pmol/min/mg). The mutation most likely lies in the structural gene encoding GlcAT-I since **transfection** of the mutant with a cDNA for GlcAT-I completely restored enzyme activity and **glycosaminoglycan** synthesis. These findings suggest that a single GlcAT effects the biosynthesis of common linkage region of both heparan sulfate and chondroitin sulfate in Chinese hamster ovary cells.

L13 ANSWER 5 OF 13 MEDLINE
ACCESSION NUMBER: 1999196236 MEDLINE
DOCUMENT NUMBER: 99196236 PubMed ID: 10098600
TITLE: Regulation of N-acetylgalactosamine 4-sulfatase expression in retrovirus-transduced feline mucopolysaccharidosis type VI muscle cells.
AUTHOR: Yogalingam G; Muller V; Hopwood J J; Anson D S
CORPORATE SOURCE: Department of Chemical Pathology, Women's and Children's Hospital, North Adelaide, South Australia, Australia.
SOURCE: DNA AND CELL BIOLOGY, (1999 Mar) 18 (3) 187-95.
Journal code: AF9; 9004522. ISSN: 1044-5498.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199904
ENTRY DATE: Entered STN: 19990420
Last Updated on STN: 20000303
Entered Medline: 19990407

AB As a preliminary step toward muscle-mediated gene therapy in the mucopolysaccharidosis (MPS) type VI cat, we have analyzed the transcriptional regulation of feline N-acetylgalactosamine 4-sulfatase (f4S) gene expression from various retroviral constructs in primary cultures of muscle cells. Two retroviral constructs were made containing the f4S cDNA under the transcriptional control of the human polypeptide chain-elongation factor 1alpha (EF1alpha) gene promoter or the cytomegalovirus (CMV) immediate-early promoter. Two further retroviral constructs were made with the murine muscle creatine kinase (mck) enhancer sequence upstream of the internal promoter. **Virus** made from each

construct was used to transduce feline MPS VI myoblasts. The mck enhancer significantly upregulated f4S gene expression from both the EF1alpha promoter and the CMV promoter in transduced myoblasts and in differentiated myofibers. The highest level of 4S activity was observed in myoblasts and myofibers transduced with the retroviral construct Lmckcmv4S, in which the f4S gene is under the transcriptional regulation of the mck enhancer and CMV immediate-early promoter. Lmckcmv4S-transduced myofibers demonstrated correction of **glycosaminoglycan** storage and contained a 58-fold elevated level of 4S activity compared with normal myofibers. Recombinant f4S secreted from Lmckcmv4S-transduced myofibers was endocytosed by feline MPS VI myofibers, leading to correction of the biochemical storage phenotype.

L13 ANSWER 6 OF 13 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 97122185 EMBASE
DOCUMENT NUMBER: 1997122185
TITLE: [Oncogenic activation of p21(ras) or pp60(c-src) in human colonic Caco-2 cells is associated with post-translational alterations of syndecan-1].
L'ACTIVATION ONCOGENIQUE DE P21(RAS) OU PP60(C-SRC) DANS LES CELLULES COLIQUES HUMAINES CACO-2 INDUIT DES ALTERATIONS POST-TRADUCTIONNELLES DU SYNDECAN-1.
AUTHOR: Levy P.; Munier A.; Baron-Delage S.; Chastre E.; Gespach C.; Capeau J.; Cherqui G.
CORPORATE SOURCE: P. Levy, INSERM U. 402, Faculte de Medecine Saint-Antoine, 27, Rue Chaligny, 75571 Paris Cedex 12, France
SOURCE: Bulletin du Cancer, (1997) 84/3 (235-237).
Refs: 9
ISSN: 0007-4551 CODEN: BUCABS
COUNTRY: France
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 005 General Pathology and Pathological Anatomy
016 Cancer
048 Gastroenterology
LANGUAGE: French
SUMMARY LANGUAGE: French; English
AB The protein encoded by ras and src protooncogenes are frequently activated in a constitutive state in human colorectal cancer. In this study, we investigated the effect of oncogenic p21(ras) and Py-MT/pp60(c-src) on the synthesis of syndecan-1, a membrane anchored proteoglycan playing a role in cell-matrix interaction and neoplastic growth control. To this end, we used Caco-2 cells transfected with an activated (Val-12) human Ha-ras gene or the polyoma middle T (Py-MT) oncogene, a constitutive activator of pp60(c-src) tyrosine kinase activity. As compared to control vector-transfected Caco-2 cells, both oncogene-transfected cells exhibited: (1) a decrease in syndecan-1 specific activity; (2) a decrease in size and sulfation of syndecan-1 ectodomain **glycosaminoglycan** side chains; and (3) an active heparanase specifically degrading the heparan sulfate chains. In conclusion, the tumorigenic progression induced by oncogenic p21(ras) or Py-MT/pp60(c-src) is associated with marked alterations of syndecan-1 at the post-translational level.

L13 ANSWER 7 OF 13 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 3
ACCESSION NUMBER: 95313200 EMBASE
DOCUMENT NUMBER: 1995313200
TITLE: Elevated levels of syndecan-1 expression confer potent serum-dependent growth in human 293T cells.
AUTHOR: Numa F.; Hirabayashi K.; Tsunaga N.; Kato H.; O'Rourke K.; Shao H.; Stechmann- Lebakken C.; Varani J.; Rapraeger A.;

Dixit V.M.
CORPORATE SOURCE: Department of Obstetrics/Gynecology, Yamaguchi Univ. School
of Medicine, 1144 Kogushi,Ube 755, Japan
SOURCE: Cancer Research, (1995) 55/20 (4676-4680).
ISSN: 0008-5472 CODEN: CNREA8
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 016 Cancer
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Syndecan-1 is the best studied integral membrane proteoglycan and functions to modulate epithelial cell attachment and physiology. Extracellularly, syndecan-1 binds both growth factors and extracellular matrix components, and intracellularly, its cytoplasmic portion interacts with cytoskeletal components. To investigate the possible role of syndecan-1 in epithelial cell transformation that is characterized by alteration in extracellular matrix interactions and cytoskeleton architecture, we established stable transfectants of syndecan-1 in a highly transformed human renal epithelial line expressing two **viral** oncogenes, adenovirus E1a and SV40 large T antigen (293T cell line). Expression of syndecan-1 core protein and appropriate posttranslational attachment of **glycosaminoglycan** chains was confirmed by enzymatic digestion and Western blot analysis. Overexpresser cells grew at a significantly faster rate than the vector-transfected control cells in serum-rich media but showed a proliferative disadvantage in serum- reduced media. In addition to this serum dependency, syndecan-1 overexpression caused a partial reversal of the transformed phenotype with the expressing clones becoming more anchorage dependent and less motile than the vector-transfected counterparts. Surprisingly, the overexpressers were more tumorigenic when injected s.c. into nude mice. These results indicate that syndecan-1 expression plays a role in the control of cell proliferation and suggest that serum-dependent growth may be the more reflective of tumorigenicity in nude mice.

L13 ANSWER 8 OF 13 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 4
ACCESSION NUMBER: 1995:870226 CAPLUS
DOCUMENT NUMBER: 123:312111
TITLE: MHC proteins and heparan sulfate proteoglycans regulate murine cytomegalovirus infection
AUTHOR(S): Price, Patricia; Allcock, Richard Jn; Coombe, Deirdre R; Shellam, Geoffrey R; McCluskey, James
CORPORATE SOURCE: Department Microbiology, University Western Australia, Nedlands, 6009, Australia
SOURCE: Immunol. Cell Biol. (1995), 73(4), 308-15
CODEN: ICBIEZ; ISSN: 0818-9641
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Factors influencing MCMV infection mediated by MHC class I mols. were analyzed further as previous studies showed that the effects of the MHC genotype on sensitivity to this **virus** are important in vivo. Here the authors show that H-2d, H-2b, H-2r, and H-2v macrophages are highly sensitive to MCMV. Moreover, **transfection** of H-2k L-cells with Kb or Dd conferred sensitivity to MCMV. This was not affected by amino acid substitutions in Kb .alpha.1 or .alpha.2, although previous studies demonstrated that exchange of the .alpha.1 domain of Dd with Ld .alpha.1 compromised sensitivity. Here replacement of Kb .alpha.3 with Ld .alpha.3 reduced susceptibility to low doses of MCMV. In addn., extracellular .beta.2-microglobulin (.beta.2m) promoted infection of .beta.2m-neg. RIE/TL8X.1 cells transfected with Db with or without a .beta.2m gene. Hence MCMV infection can involve .beta.2m and the .alpha.1

and .alpha.3 domains of MHC heavy chains. MCMV infection of L-cells expressing Dd or Kb was also inhibited by heparin, but infection of the parental L-cell line was not reproducibly affected. A role for heparan sulfate proteoglycan in MHC-mediated MCMV infection was confirmed using cells pre-treated with heparinase I or III, or propagated in chlorate to inhibit the sulfation of the **glycosaminoglycan** chains.

L13 ANSWER 9 OF 13 MEDLINE
ACCESSION NUMBER: 94118394 MEDLINE
DOCUMENT NUMBER: 94118394 PubMed ID: 8289356
TITLE: Herpesvirus-induced cell fusion that is dependent on cell surface heparan sulfate or soluble heparin.
AUTHOR: Shieh M T; Spear P G
CORPORATE SOURCE: Department of Microbiology-Immunology, Northwestern University Medical School, Chicago, Illinois 60611.
CONTRACT NUMBER: CA 21776 (NCI)
SOURCE: JOURNAL OF VIROLOGY, (1994 Feb) 68 (2) 1224-8.
Journal code: KCV; 0113724. ISSN: 0022-538X.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199402
ENTRY DATE: Entered STN: 19940312
Last Updated on STN: 19940312
Entered Medline: 19940218

AB The entry of enveloped **viruses** into animal cells and the cell-to-cell spread of infection via cell fusion require the membrane-fusing activity of **viral** glycoproteins. This activity can be dependent on variable cell factors or triggered by environmental factors. Here we show that cell fusion induced by herpes simplex **virus** glycoproteins is dependent on the presence of cell surface glycosaminoglycans, principally heparan sulfate, or on the addition of heparin to the medium. The role of the **glycosaminoglycan** is probably to alter the conformation of a **viral** heparin-binding glycoprotein required for the fusion.

L13 ANSWER 10 OF 13 MEDLINE
ACCESSION NUMBER: 93393540 MEDLINE
DOCUMENT NUMBER: 93393540 PubMed ID: 8379921
TITLE: Overexpression of N-acetylgalactosamine-4-sulphatase induces a multiple sulphatase deficiency in mucopolysaccharidosis-type-VI fibroblasts.
AUTHOR: Anson D S; Muller V; Bielicki J; Harper G S; Hopwood J J
CORPORATE SOURCE: Department of Chemical Pathology, Adelaide Children's Hospital, South Australia.
SOURCE: BIOCHEMICAL JOURNAL, (1993 Sep 15) 294 (Pt 3) 657-62.
Journal code: 9YO; 2984726R. ISSN: 0264-6021.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199310
ENTRY DATE: Entered STN: 19931105
Last Updated on STN: 19931105
Entered Medline: 19931019

AB High-titre stocks of an amphotropic retrovirus, constructed so as to express a full-length cDNA encoding the human lysosomal enzyme N-acetylgalactosamine-4-sulphatase (4-sulphatase) from the cytomegalovirus immediate early promoter, were used to infect skin fibroblasts from a clinically severe mucopolysaccharidosis type VI (MPS VI) patient. The infected MPS VI cells showed correction of the enzymic defect with the enzyme being expressed at high levels and in the correct subcellular compartment. Surprisingly this did not result in correction of **glycosaminoglycan** turnover as measured by accumulation of 35S in

metabolically labelled cells. We demonstrate that this is apparently caused by an induced reduction of the activities of other lysosomal sulphatases, presumably due to competition for a sulphatase-specific processing mechanism by the over-expressed 4-sulphatase. The level of steroid sulphatase, which is a microsomal sulphatase, was also reduced. Infection of skin fibroblasts from a second, clinically mildly affected, MPS VI patient with the same **virus** also resulted in no significant change in the level of **glycosaminoglycan** storage. However, in this case the cause of the observed phenomenon was less clear.

These results are of obvious practical importance when considering gene therapy for a sulphatase deficiency such as MPS VI and also provide possible new avenues for exploration of the processes involved in sulphatase synthesis and genetically determined multiple sulphatase deficiency.

L13 ANSWER 11 OF 13 MEDLINE
ACCESSION NUMBER: 93066238 MEDLINE
DOCUMENT NUMBER: 93066238 PubMed ID: 1279676
TITLE: T-cell activation molecule 4-1BB binds to extracellular matrix proteins.
AUTHOR: Chalupny N J; Peach R; Hollenbaugh D; Ledbetter J A; Farr A
G; Aruffo A
CORPORATE SOURCE: Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle, WA 98121.
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1992 Nov 1) 89 (21) 10360-4. Journal code: PV3; 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199212
ENTRY DATE: Entered STN: 19930122
Last Updated on STN: 19970203
Entered Medline: 19921211

AB The recently isolated 4-1BB cDNA clone encodes a cell surface protein expressed by activated T cells. Its extracellular domain is homologous to members of the nerve growth factor receptor super family and its cytoplasmic domain contains a sequence homologous to the binding site for the T-cell-specific tyrosine kinase p56lck found in the cytoplasmic domains of CD4 and CD8 alpha. At present the function of 4-1BB is not known. We prepared a 4-1BB-immunoglobulin fusion protein (4-1BB Rg). This protein was used in immunohistochemical studies to identify tissues that express the 4-1BB ligand. 4-1BB Rg bound to **virtually** all tissues examined, suggesting that extracellular components might function as its ligands. To explore this possibility, 4-1BB was expressed in COS cells and found to mediate the binding of fibronectin, vitronectin, laminin, and collagen VI but not of collagen I. The binding of extracellular matrix proteins to 4-1BB was not mediated by Arg-Gly-Asp (RGD) or CS-1 amino acid sequences. Experiments with overlapping proteolytic fragments of fibronectin showed that 4-1BB interacts with multiple regions of fibronectin. The interaction between extracellular matrix proteins and 4-1BB was completely blocked by the anionic carbohydrate polymer fucoidan and was partially blocked by the anionic carbohydrate polymer dextran sulfate and the **glycosaminoglycan** heparin sulfate but was unaffected by desulfated heparin. These results suggest that carbohydrates may play a role in mediating the 4-1BB-extracellular matrix protein adhesion.

L13 ANSWER 12 OF 13 MEDLINE
ACCESSION NUMBER: 92399510 MEDLINE
DOCUMENT NUMBER: 92399510 PubMed ID: 1525210
TITLE: Correction of mucopolysaccharidosis type I fibroblasts by retroviral-mediated transfer of the human

alpha-L-iduronidase gene.
AUTHOR: Anson D S; Bielicki J; Hopwood J J
CORPORATE SOURCE: Department of Chemical Pathology, Adelaide Children's Hospital, South Australia.
SOURCE: HUMAN GENE THERAPY, (1992 Aug) 3 (4) 371-9.
Journal code: A12; 9008950. ISSN: 1043-0342.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199210
ENTRY DATE: Entered STN: 19921106
Last Updated on STN: 19921106
Entered Medline: 19921020

AB Three retroviral constructs containing a full-length human alpha-L-iduronidase (IDUA) cDNA were made. The first, pLIdSN, is designed so that expression of the IDUA cDNA is from the 5' **viral** long terminal repeat (LTR). The second, pLNCId, is designed to express the IDUA cDNA from the cytomegalovirus (CMV) immediate early promoter, while in the third, pLNTId, the CMV promoter is replaced by a promoter fragment of the mouse CD45 (T200) gene. All vectors transduce resistance to G418 (neomycin). High-titer **virus**-producing cell lines for these constructs were made by infection of the amphotropic packaging cell line PA317 after transient expression in, and **virus** rescue from, the ecotropic packaging cell line psi CRE. The high-titer **virus**-producing cell lines were assayed for absence of helper **virus**, synthesis of human IDUA, and for integrity of proviral structure. Suitable lines were used as a source of **virus** to infect two different mucopolysaccharidosis type I (MPS I) skin fibroblast cultures. All three of the recombinant **viruses** corrected the enzymatic defect in MPS I fibroblasts. Surprisingly, increasing over-expression of IDUA resulted in reduced phenotypic correction of these cells as assayed by intracellular accumulation of 35S-labeled **glycosaminoglycan**. This was shown to be due to the induction of a phenotype analogous to mild I-cell disease in cells expressing large amounts of IDUA.

L13 ANSWER 13 OF 13 MEDLINE
ACCESSION NUMBER: 90384933 MEDLINE
DOCUMENT NUMBER: 90384933 PubMed ID: 2144898
TITLE: Ligand-affinity cloning and structure of a cell surface heparan sulfate proteoglycan that binds basic fibroblast growth factor.
AUTHOR: Kiefer M C; Stephans J C; Crawford K; Okino K; Barr P J
CORPORATE SOURCE: Chiron Corporation, Emeryville, CA 94608.
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1990 Sep) 87 (18) 6985-9.
Journal code: PV3; 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-M29967
ENTRY MONTH: 199010
ENTRY DATE: Entered STN: 19901122
Last Updated on STN: 19980206
Entered Medline: 19901024

AB Expression cloning of cDNAs encoding a basic fibroblast growth factor (FGF) binding protein confirms previous hypotheses that this molecule is a cell-surface heparan sulfate proteoglycan. A cDNA library constructed from a hamster kidney cell line rich in FGF receptor activity was transfected

into a human lymphoblastoid cell line. Clones expressing functional basic FGF binding proteins at their surfaces were enriched by panning on plastic dishes coated with human basic FGF. The amino acid sequence deduced from the isolated cDNAs revealed several interesting features, including hydrophobic signal and transmembrane domains that flank an extracellular region containing six potential attachment sites for **glycosaminoglycan** side chains. The structure also contains a short hydrophilic cytoplasmic tail sequence homologous to previously reported actin binding domains. Binding of basic FGF to cells expressing the binding protein could be inhibited by heparin and heparan sulfate but not by chondroitin sulfate, dermatan sulfate, or keratan sulfate. In addition to binding basic FGF, this protein or related surface proteins may function as an initial cellular attachment site for other growth factors and for **viruses**, such as herpes simplex **virus**.

drewwang

=> s (dextran sulfate) and transfection

L14 54 (DEXTRAN SULFATE) AND TRANSFECTION

=> s l14 and (gene therapy)

L15 4 L14 AND (GENE THERAPY)

=> dup rem l15

PROCESSING COMPLETED FOR L15
L16 4 DUP REM L15 (0 DUPLICATES REMOVED)

=> d 1- ibib abs

YOU HAVE REQUESTED DATA FROM 4 ANSWERS - CONTINUE? Y/(N):y

L16 ANSWER 1 OF 4 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1999:762507 CAPLUS
DOCUMENT NUMBER: 132:132916
TITLE: Apoptosis Induced by DNA Uptake Limits **Transfection** Efficiency
AUTHOR(S): Li, L. H.; Sen, A.; Murphy, S. P.; Jahreis, G. P.; Fuji, H.; Hui, S. W.
CORPORATE SOURCE: Department of Cellular & Molecular Biophysics, Roswell Park Cancer Institute, Buffalo, NY, 14263, USA
SOURCE: Exp. Cell Res. (1999), 253(2), 541-550
CODEN: ECREAL; ISSN: 0014-4827
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Electrotransfection is an effective method for transfecting lymphoid cells. However, the **transfection** efficiency of certain lymphoid cells is low. L1210 subclones and NFS-70 pro-B cells, which are highly refractory to various **transfection** methods, were used to identify the limiting factors. Cells were electrotransfected with plasmids coding for green fluorescence protein or luciferase. The luciferase expression of L1210 subclone 3-3 was found to increase 6-12 h after electroporation, but decreased significantly from 12 to 48 h. The lower level of luciferase activity at later time periods correlated with decreases in cell viability, which was shown to be due to apoptosis, as detd. by propidium iodide/acrindine orange staining, DNA laddering, and prevention of cell death by addn. of caspase inhibitors. Similar results were obsd. with NFS-70 pro-B cells and select L1210 subclones. In contrast, L1210 parental and L1210 subclone 7-15.6 cells undergo only low levels of apoptosis (.ltoreq.5%). Apoptosis occurred only when DNA (plasmids or salmon sperm DNA) was present during electroporation, but was not dependent on the conformation of the DNA used or the expression of transgenes. Cells pulsed in the presence of **dextran sulfate** (MW 500,000) did not apoptose. Similar results were obsd. when L1210 subclone 3-3 was transfected using the cationic lipid 1,2-dioleoyl-3-trimethylammonium propane, although the **transfection** efficiency and corresponding rate of apoptosis were significantly lower. Applying the caspase inhibitor fluoromethyl ketone (Boc-ASP-FMK) dramatically improved cell viability and transgene

expression of select L1210 subclones and NFS-70 pro-B cells. (c) 1999 Academic Press.

REFERENCE COUNT: 47

REFERENCE(S):
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(6) Chittenden, T; Nature 1995, V374, P733 CAPLUS
(7) Cron, R; J Immunol Methods 1997, V205, P145 CAPLUS

ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 2 OF 4 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1999:700825 CAPLUS

DOCUMENT NUMBER: 132:69209

TITLE: Pharmaceutical and biological properties of poly(amino acid)/DNA polyplexes

AUTHOR(S): Lucas, P.; Milroy, D. A.; Thomas, B. J.; Moss, S. H.; Pouton, C. W.

CORPORATE SOURCE: Department of Pharmacy and Pharmacology, University of Bath, Bath, BA2 7AY, UK

SOURCE: J. Drug Targeting (1999), 7(2), 143-156
CODEN: JDTAEH; ISSN: 1061-186X

PUBLISHER: Harwood Academic Publishers

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Physicochem. properties of polyplexes formed between pRSVlacZ and poly(amino acid)s were investigated as a paradigm of more complex, synthetic virus-like, DNA delivery systems, that are of interest to many gene delivery labs. We obsd. the interaction between polymer and DNA using ethidium exclusion, and detd. the size distributions and the zeta potentials of polyplexes. We correlated these properties with their fundamental interactions with cultured B16 murine melanoma cells, and the resulting efficiency of **transfection**. A variety of poly(amino acid)s each condensed DNA to produce particles with mean hydrodynamic diams. of approx. 100 nm (a typical span of a population was 80-120 nm). Poly(amino acid) polyplexes were unstable in electrolyte solns. such as cell culture media. The apparent particle size increased in electrolyte, depending on the charge ratio, to diams. up to 700 nm. This was thought to be due to aggregation, since neutral particles were most sensitive. When the charge ratio (+/-) exceeded unity polyplexes had pos. zeta potentials (which peaked at approx. +30 mV), bound non-specifically to cells, were internalized and in the presence of an endosomolytic agent were able to transfect cells. Though all cationic poly(amino acid)s investigated formed polyplexes with similar phys. properties, their biol. properties were significantly different. Polyplexes prepd. with poly-L-ornithine were the most effective **transfection** agents, but poly(lys-co-ala, 1: 1) systems appeared to be inactive. This may reflect the differences in uncoupling of DNA and polymer, which is expected to be necessary for passage through the nuclear pore. Uncoupling of polycation and DNA was investigated by exposing the complexes to **dextran sulfate**. Release of DNA was detected by increased fluorescence at 600 nm in the presence of ethidium. Release of DNA was incomplete from polyplexes formed with high mol. wt. polylysine. This may explain the lower levels of **transfection** obsd. with high mol. wt. polylysine. The significance of these observations for design of advanced non-viral gene delivery systems is discussed.

REFERENCE COUNT: 44

REFERENCE(S):
(1) Alton, E; Nat Genet 1993, V5, P135 CAPLUS
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FILE SEGMENT: Priority Journals
ENTRY MONTH: 199607
ENTRY DATE: Entered STN: 19960726
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AB To understand how DNA is released from cationic liposome/DNA complexes in cells, we investigated which biomolecules mediate release of DNA from a complex with cationic liposomes. Release from monovalent[1,2-dioleoyl-3(1)-1(trimethylammonio)propane] or multivalent (dioctadecylamidoglycylspermine) lipids was quantified by an increase of ethidium bromide (EtBr) fluorescence. Plasmid sensitivity to DNAse I degradation was examined using changes in plasmid migration on agarose gel electrophoresis. Physical separation of the DNA from the cationic lipid was confirmed and quantified on sucrose density gradients. Anionic liposomes containing compositions that mimic the cytoplasmic-facing monolayer of the plasma membrane (e.g. phosphatidylserine) rapidly released DNA from the complex. Release occurred near a 1/1 charge ratio (-/+) and was unaffected by ionic strength or ion type. Water soluble molecules with a high negative linear charge density such as **dextran sulfate** or heparin also released DNA. However, ionic water soluble molecules such as ATP, tRNA, DNA, poly(glutamic acid), spermidine, spermine, or histone did not, even at 100-fold charge excess (-/+). On the basis of these results, we propose that after the cationic lipid/DNA complex is internalized into cells by endocytosis it destabilizes the endosomal membrane. Destabilization induces flip-flop of anionic lipids from the cytoplasmic-facing monolayer, which laterally diffuse into the complex and form a charge neutral ion pair with the cationic lipids. This results in displacement of the DNA from the cationic lipid and release of the DNA into cytoplasm. This mechanism accounts for a variety of observations on cationic lipid/DNA complex-cell interactions.

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